Aporphines. 48.[1] Enantioselectivity of (*R*)-(-)- and (*S*)-(+)-*N*-*n*-Propylnorapomorphine on Dopamine Receptors[2]

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The enantiomers [(*S*)-(+) and (*R*)-(-)] of *N*-*n*-propylnorapomorphine (NPA) were synthesized. (*R*)-NPA was obtained by the acid-catalyzed rearrangement of *N*-*n*-propylnormorphine. (*R*)-NPA also was converted to (*RS*)-*N*-*n*-propylnorapomorphine dimethyl ether by dehydrogenation of the 10,11-*O*,*O*'-dimethyl ether of (*R*)-NPA with 10% palladium on carbon in acetonitrile, followed by reduction with sodium cyanoborohydride under acidic conditions. Alternatively (*RS*)-NPA 10,11-*O*,*O*'-dimethyl ether was obtained via total synthesis. (+)-Dibenzoyl-D-tartaric acid was used to resolve (*RS*)-NPA dimethyl ether. Ether cleavage gave (*S*)-NPA isolated as the hydrochloride salt in greater than 99.9% enantiomeric purity, as determined by circular dichroism (CD) spectra. The pharmacological activities of (*S*)- and (*R*)-NPA were evaluated with subnanomolar concentrations of ^{3}H-labeled apomorphine (APO), ADTN, and spiroperidol (SPR) for competition for binding to a membrane-rich subsynaptosomal fraction of calf caudate nucleus. IC_{50} (nM) values for (*R*)-NPA vs. (*S*)-NPA were as follows: [^{3}H]APO, 2.5 vs. 66; [^{3}H]ADTN, 2.0 vs. 60; [^{3}H]SPR, 174 vs. 1400. The efficacy of (*R*)- and (*S*)-NPA in stimulating dopamine-sensitive adenylate cyclase from both homogenates of rat corpus striatum and pieces of intact carp retina was also evaluated. Three behavioral effects in the rat (stereotyped behavior, sedation, and catalepsy) were also examined. Only (*R*)-NPA induced stereotypy; (*S*)-NPA failed to antagonize this action of the *R* isomer. The effects of (*R*)- and (*S*)-NPA on adenylate cyclase agreed with the behavioral effects and radioreceptor binding assays in that the *R* isomer was the strongly preferred enantiomer at dopamine receptors. The *S* enantiomer of NPA was, however, the weakly preferred configuration for rat liver catechol *O*-methyltransferase. A dopamine-receptor model accommodates the configuration of NPA and related aporphines.

The recent recognition of the importance of dopamine (DA) as a neurotransmitter at receptors in the central nervous system (CNS) and at several sites in the peripheral nervous system has stimulated intense interest in the pharmacology of DA receptors or sites of probable interactions of DA and its agonists and antagonists. The search for agents that may selectively exert antipsychotic effects in cortical and limbic regions or neurological effects on the basal ganglia is of great current interest.[3] One potential strategy is the development of DA agents selective for presynaptic or autoreceptors. For example, beneficial effects of low doses of apomorphine (APO) and *N*-*n*-propylnorapomorphine (NPA) have been reported against psychosis and dyskinesia.[4-8] These seemingly paradoxical antipsychotic or antidyskinetic effects of DA agonists are consistent with the hypothesis that DA autoreceptors may decrease dopaminergic transmission by decreasing the synthesis and release of DA.[9] The recent realization that (*R*)-*N*-*n*-propylnorapomorphine [(*R*)-NPA, (*R*)-(-)-2] is an extremely potent agonist of DA autoreceptors in vivo[10] suggested its comparison with the enantiomer [(*S*)-NPA, (*S*)-(+)-2] for DA-like activity to further advance our understanding of central DA receptors.

(*R*)-(-)-APO, the enantiomer obtained by the acid-catalyzed rearrangement of morphine, was reported by Saari et al.[11] to be the active enantiomer for dopaminergic and emetic activity. The S enantiomer, prepared by the resolution of (±)-apomorphine dimethyl ether,[12] was shown at Merck in 1973 to be inactive in producing postural asymmetries in unilateral caudate-lesioned mice. Recently, Riffee et al.[13] reported that (*S*)-APO possessed no agonistic activity at 30 and 50 mg/kg sc compared to (*R*)-APO, which showed an ED_{50} of 4.3 mg/kg in inducing stereotyped cage climbing in mice. Enantiomers of other dopaminergic agents, such as the aporphines [(*R*)- and (*S*)-2,10,11-trihydroxyaporphine[14] and (*S*)- and (*R*)-1,2-dihydroxyaporphine[15]] and the hydroxylated 2-amino-1,2,3,4-tetrahydronaphthalene (aminotetralin) derivatives,[16] have been utilized to complement structure–activity

Scheme I. Synthesis of (*R*) and (*S*)-*N*-*n*-Propylnorapomorphine

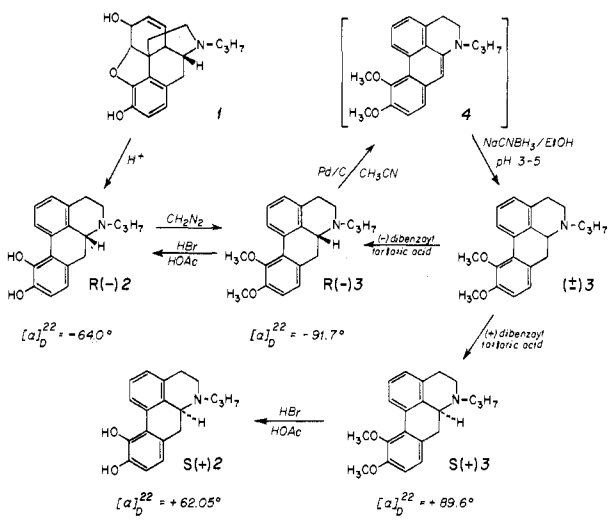

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Table I. Behavioral Effects of Isomers of NPA in the Rat[a]

	stereotypy scores		general act (EAM counts)		catalepsy scores	
dose, mg/kg	(R)-(-)-NPA	(S)-(+)-NPA	(R)-(-)-NPA	(S)-(+)-NPA	(R)-(-)-NPA	(S)-(+)-NPA
0	0	0	380 ± 46	380 ± 46	0	0.4 ± 0.01
0.1	6.3 ± 0.8	0	386 ± 52	392 ± 71	0.5 ± 0.2	0.6 ± 0.1
0.3	12.0 ± 1.2	0	460 ± 0.1	360 ± 81	nd	0.4 ± 0.1
3.0	17.2 ± 0.5	0	1260 ± 290	190 ± 22	0.8 ± 0.3	0.6 ± 0.2
5.0	18.0 ± 0	0	nd	182 ± 26	nd	0.6 ± 0.3
10	18.0 ± 0	0	1502 ± 262	203 ± 18	0.4 ± 0.1	11.8 ± 1.8

[a] Behaviors were evaluated as described under Experimental Section; all scores are means plus or minus SEM (doses all sc) for four to six rats per dose. The results indicate a dose-dependent induction of stereotypy by (R)-(-)-NPA only. There was a dose-dependent increase of electronically detected general motor activity (EAM) but no induction of catalepsy by doses of (R)-(-)-NPA up to 10 mg/kg. (S)-(+)-NPA induced moderate (mean = 50 ± 5%) inhibition of EAM counts that was *not* dose dependent at doses of 3 to 10 mg/kg. (S)-(+)-NPA also induced moderate catalepsy at the highest dose tested only. nd = doses not tested.

relationships in probing DA receptors. The enantiomeric and conformational selectivity of aporphine and aminotetralin derivatives in binding to DA receptors has led to several conceptual models that suggest possible binding sites and boundaries of the DA receptor.[16-19]

In this report, the separation of the enantiomers of NPA is described, together with a comparison of their dopaminergic and other actions in several biochemical and behavioral test systems. The stereochemistry and conformation of NPA, as they relate to our previous observations of the structural requirements for binding with central DA receptors, contribute further to our proposed model of the surface of DA agonist binding sites or "receptors" as is discussed below.

Chemistry. The acid-catalyzed rearrangement of morphine or *N*-*n*-propylnormorphine (1) is a well-established method for obtaining (*R*)-apomorphine[20] or (*R*)-*N*-*n*-propylnorapomorphine [(*R*)-(-)-**2**].[21] The conversion of **2** to its dimethyl ether (**3**) was accomplished with diazomethane by a published method.[22] The 10,11-*O*,*O*′-dimethyl ether of NPA, (±)-**3**, was obtained by a multistep procedure from vanillin and isoquinoline[22] (Scheme I).

We found that (+)-dibenzoyl-D-tartaric acid was a convenient resolving agent for the resolution of (*RS*)-**3**; after three recrystallizations from a mixture of EtOAc/2-propanol, a diastereoisomeric salt with a constant specific rotation was obtained. Ether cleavage with HBr[22] yielded (*S*)-**2**, which, by gradient HPLC [15 cm × 4.6 mm Supelcosil LC-18 column: exponential gradient from 30% acetonitrile/70% buffer to 80% acetonitrile/20% buffer over a 10-min period at a flow rate of 2 mL/min], indicated a product of greater than 99% chemical purity. The circular dichroism (CD) spectra of (*S*)-**2**·HCl and (*R*)-**2**·HCl are shown in Figure 1. The most intense extremes for the Cotton effect were observed at 232 nm with molar ellipticies of −25.9 and +25.8 × 10^{-4} units for *R* and *S* configurations, respectively. The *R*/*S* ratio is 1.004, which suggests the relative enantiomeric purity of the two isomers of *N*-*n*-propylnorapomorphine utilized for our biological studies.

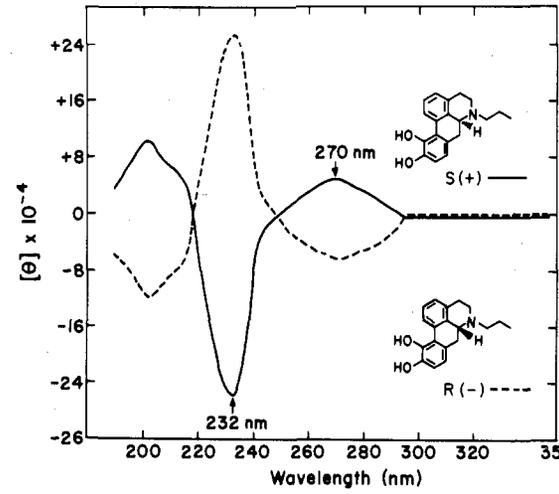

Figure 1. CD spectra of *N*-*n*-propylnorapomorphines.

Table II. Interactions of (*R*)-(+)- and (*S*)-(-)-Aporphine Enantiomers on Rat Stereotyped Behavior[a]

treatment	stereotypy scores
(R)-(-)-NPA + saline	17.6 ± 0.2
(R)-(-)-APO + saline	18.0 ± 0
(S)-(+)-NPA + saline	0
(S)-(+)-NPA + (R)-(-)-NPA	17.2 ± 0.8
(S)-(+)-NPA + (R)-(-)-APO	17.5 ± 0.2
(R)-(-)-NPA + (S)-(+)-NPA	18.0 ± 0
(R)-(-)-APO + (S)-(+)-NPA	17.7 ± 0.3

[a] (*S*)-(+)-NPA, 5 mg/kg sc, was given 30 min before or 30 min after (*R*)-(-)-NPA or (*R*)-(-)-APO (both at 3 mg/kg sc). Stereotypy scores were measured in the 60 min following (*R*)-(-)-NPA or (*R*)-(-)-APO. Data are mean scores plus or minus SEM (n = 4); there were no significant differences from the control condition for (*R*)-(-)-NPA or (*R*)-(-)-APO alone.

The combined mother liquors containing enriched (*R*)-**3**, obtained after resolution of (*S*)-**3**, when treated with (-)-dibenzoyl-L-tartaric acid, led to the isolation of (*R*)-**3**·HCl. The racemization of (*R*)-**3** to (*RS*)-**3** was accomplished with 10% palladium on carbon in acetonitrile according to the method of Cava et al.[23] Reducton of the dehydroaporphine **4** with $NaCNBH_3$ in EtOH at pH 5 by modifications of the method used by Davis et al.[24] for the

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Table III. Affinity of Isomers of *N*-*n*-Propylnorapomorphine (NPA) for Dopamine Receptors[a]

ligand (nM)	IC_{50}, nM (*R*)-(−)-NPA	(±)-NPA	(*S*)-(+)-NPA
[^{3}H]APO (0.5)	2.5	5.0	66
[^{3}H]ADTN (0.5)	2.0	3.5	60
[^{3}H]SPR (0.15)	174	670	1414

[a] Fractions (P_4) of caudate nucleus from calf brain were prepared and incubated with low concentrations of tritiated apomorphine (APO), ADTN, or spiroperidol (SPR) as ligands in competition with a range of concentrations of the NPA isomers indicated, as described under Experimental Section. Values of IC_{50} were computed from log probit analyses of data from at least three determinations involving at least four concentrations of each aporphine. The tabulated values are means, the SD of which were ±5% or less.

racemization of (*R*)-apomorphine dimethyl ether provided an alternative method for the synthesis of the *S* antipode rather than the de novo synthesis also used for the preparation of (*RS*)-**3**.

Pharmacology. Behavioral Methods. Male Sprague–Dawley rats (Charles River Labs) (175–200 g) were housed four per cage, with free access to food and water, under controlled lighting (on, 7:00 a.m. to 7:00 p.m.), constant temperature (21–23 °C), and controlled humidity (40–50%). Aporphines were administered, as described below, freshly dissolved in ice-cold 1 mM citric acid mixed with 0.9% (w/v) saline (1:4, v/v); this solvent was also used as a "placebo" control injection.

Stereotyped behavior was evaluated by an experienced observer according to a rating scale method reported previously.[25] Briefly, the ratings were as follows: 0, no stereotypy, normal locomotion; 1, discontinuous sniffing, reduced locomotion; 2, continuous sniffing, only periodic exploration; 3, continuous sniffing, mouth movements, infrequent exploratory activity. Ratings were based on 30-s observations made each 10 min, typically for a total of 60 min (maximum score = 18.0) (Tables I and II).

Other behaviors were also assessed. **General motor activity** was evaluated by use of a printing electronic activity monitor (EAM, Stoelting Co., Chicago, IL) within a sound-attenuated chamber, typically for 60 min, as described previously.[26] **Catalepsy** was assessed as described in detail elsewhere.[27] Briefly, rats were evaluated every 10 min by timing (stopwatch) their maintenance of an abnormal posture, with forelimbs on a 1-cm diameter steel bar parallel to and 8 cm above the bench, so that the rat rested on its hindquarters only; 60 s was taken as a maximum, and nearly all normal untreated rats remained on the bar for less than 5 s. Ratings were then made as follows: 0, remaining on the bar for 0–10 s; 1, remaining for 10–29 s; 2, 30–59 s; 3, for 60 s or longer. Thus, in a typical 60-min session, the maximum attainable score was 18.0.

Binding assays were carried out with a membrane fraction (P_4) recovered from osmotically shocked and Polytron-disrupted "synaptosomal" preparations of rapidly frozen protease-free calf caudate nuclei. Ligands included

Table IV. Stimulation of Formation of Cyclic AMP in Rat Striatal Homogenates[a]

addition	concn, μM	cAMP production (*N*)	% of control
blank	0	2.48 ± 0.15 (12)	100
DA	50	5.39 ± 0.19 (12)	217
(*R*)-(−)-NPA	1	3.71 ± 0.31 (6)	150
	5	4.57 ± 0.13 (10)	184
	10	5.34 ± 0.27 (12)	215
	50	5.71 ± 0.24 (12)	230
	100	5.56 ± 0.24 (5)	224
(*S*)-(+)-NPA	10	2.95 ± 0.19 (12)	119
	100	3.19 ± 0.16 (6)	129

[a] Stimulation of the formation of cyclic AMP (cAMP) with isomers of NPA was assayed in homogenates of corpus striatum obtained from rat brain. The method of assay of the activity of DA-sensitive adenylate cyclase by the rate of formation of cAMP above a basal (no agonist) level is described under Experimental Section. The data are mean values of cAMP levels in picomoles per 2.5 min of assay plus or minus SEM. The concentration that increased cAMP by 50% (EC_{50}) is ca. 1 μM for (*R*)-(−)-NPA and much higher than 100 μM for (*S*)-(+)-NPA [potency ratio >100 in favor of the (*R*)-(−) isomer].

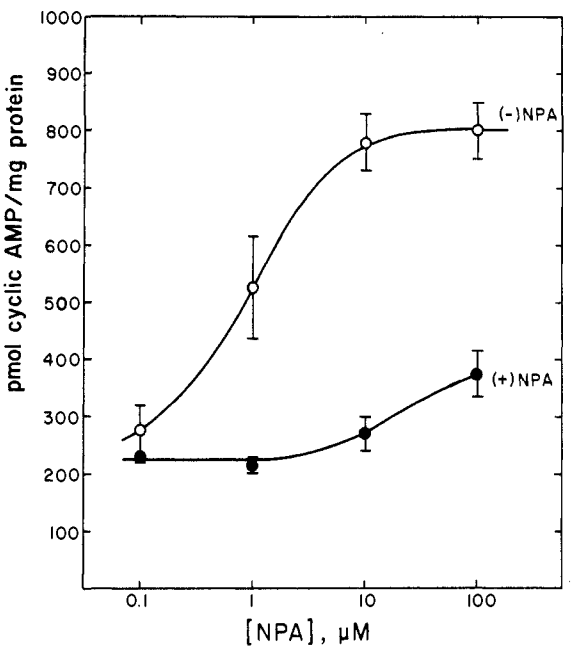

Figure 2. Effects of (*R*)-(−)-NPA (○) and (*S*)-(+)-NPA (●) on cyclic AMP (cAMP) production in intact pieces of carp retina. Retinas were prepared as described previously[32] and incubated for 5 min in Ringer's solution containing 2 mM isobutylmethylxanthine (IBMX) and for an additional 10 min in IMBX–Ringer's buffer containing either (*R*)-(−)-NPA or (*S*)-(+)-NPA. After the tissue reaction mixture was boiled, cAMP was determined in the supernatant by a protein-binding assay as described under Experimental Section and reported in detail previously.[32] Each point is the mean (±SEM) of four to eight experiments involving triplicate determinations. Data are expressed as picomoles of cAMP per milligram of protein. Control values in these experiments were 244.6 ± 11.2 pmol of cAMP/mg of protein.

(*R*)-[8,9-^{3}H]apomorphine ([^{3}H]APO; New England Nuclear, 20–30 Ci/mmol), (±)-[5,8-^{3}H]-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene ([^{3}H]ADTN; New England Nuclear, 36 Ci/mmol), and [benzene ring-^{3}H]-spiroperidol ([^{3}H]SPR, New England Nuclear, 30 Ci/mmol). Blanks were defined by adding excess (10 μM) unlabeled (±)-ADTN in the [^{3}H]APO assays, APO in the [^{3}H]ADTN assay, and (+)-butaclamol (gift of Ayerst Laboratories, New York) with [^{3}H]SPR. Further details

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Table V. Isomeric Preference of Rat Liver COMT for NPA as Substrate[a]

agent	K_m, μM	V_{max}, (pmol/assay)/h
(S)-(+)-NPA	0.48	11.7
(R)-(-)-NPA	0.94	10.0

[a] Data are mean values obtained by kinetic analyses of double-reciprocal plots of (reaction velocity)$^{-1}$ vs. (substrate concentration)$^{-1}$ with (-)-NPA and (+)-NPA as substrates, to allow computation of values for apparent K_d (negative slope) and apparent V_{max} (y intercept). Two separate experiments were carried out as described under Experimental Section with six substrate concentrations assayed in triplicate. The ranges of values for V_{max} were 8.9-11.1 vs. 10.2-13.2 and for K_d were 0.93-0.95 vs. 0.41-0.55 for (R)-(-)- vs. (S)-(+)-NPA, respectively. These results indicate a somewhat higher apparent affinity for the (S)-(+)- isomer (in contrast to results pertinent to DA receptors) but little difference in velocity of methylation at saturating substrate concentrations.

are provided in the legend to Table III and elsewhere.[28-30]

Dopamine (DA) sensitive adenylate cyclase activity was evaluated with homogenates of rat corpus striatum[31] and intact pieces of carp retina[32] by similar methods. The ability of aporphines to stimulate the accumulation of 3',5'-cyclic adenosine monophosphate (cAMP) was evaluated in striatal tissue by a protein-binding assay for cAMP (also used for the fish retina cAMP assays) after incubation of the cyclase-rich tissue in the presence of excess ATP and theophylline (rat) or IBMX (fish) to inhibit phosphodiesterase.[31,32] Typical basal activity (DA agonist omitted) (±SEM) with rat striatum was 2.48 ± 0.15 pmol of cAMP formed per 2.5 min assay, and with the fish retinal tissue, 245 ± 11 pmol/mg of protein/10 min. Results are provided for rat striatum in Table IV and for fish retina in Figure 2. Further details of the carp retina adenylate cyclase assay are given in the legend to Figure 2.

Isomeric Substrate Selectivity for COMT. Substrate selectivity for the enantiomers of NPA was tested with partially purified rat liver catechol O-methyltransferase (COMT). A range of six concentrations of substrate (in triplicate) was tested by incubation with [*methyl*-^{3}H]S-adenosyl-L-methionine (SAMe; New England Nuclear, 15.3 Ci/mmol). The enzyme was partially purified by ammonium sulfate precipitation and dialysis. The methylated product was recovered selectively in hexane and counted. The methods of purification, assay, and product recovery were essentially those described by Kebabian[33] for methylation of APO to apocodeine. The product obtained with NPA was found to migrate on silica gel TLC and high-voltage paper electrophoresis as a single peak identical in R_f with a sample of authentic (±)-N-n-propylnorapocodeine.[22] In addition, the product that formed from [^{3}H]SAMe with unlabeled NPA and extracted into hexane gave a single radioactive peak in silica gel TLC chromatography with a solvent mixture of toluene/isoamylol (3:2, v/v). The product R_f (0.79) was clearly separated in a single peak from the labeled cofactor (at origin). Kinetic data were evaluated by linear transformation of the data [(velocity of product formation)$^{-1}$ vs. (substrate concentration)$^{-1}$] to obtain values for apparent affinity of substrate (K_d = negative slope) and maximum reaction velocity (V_{max} = y intercept). The results are provided in Table V.

Results and Discussion

When the enantiomers of NPA were evaluated in various tests that presumably indicate their interactions with dopamine (DA) receptors of the central nervous system (CNS), there was a clear preference for the R optical isomer. This conclusion is strongly supported by the differences in potency for the preparations of the optically active enantiomers as well as those of the racemate vs. the binding of two tritiated DA agonist ligands (APO and ADTN) and a potent and selective DA antagonist (SPR) (Table III). The isomeric difference across all three ligand binding assays averaged 2.53 for the comparison of (R)- vs. (RS)-NPA and averaged 21.5 for (R)- vs. (S)-NPA. In both comparisons, the results in the spiroperidol binding assays were somewhat aberrant in that the potency difference for (R)- vs. racemic NPA was greater than 2 (3.8), while the comparison of (R)- vs. (S)-NPA revealed only a 8.1-fold potency difference. The apparent inconsistency of results between agonist and an antagonist binding assay may reflect the much lower affinity of NPA for DA-antagonist sites. While the qualitative comparison of the enantiomers is strongly indicative of the preference for (R)-NPA at central DA receptors, the finding of only a 22-fold potency difference for (R)- vs. (S)-NPA, on average, at first suggested that the latter preparation might possibly contain traces of (R)-NPA, since the chemical preparation of (S)-NPA involved the resolution of a racemic intermediate. In contrast, for example, we found a 500-fold difference in potency favoring (R)-2,10,11-trihydroxyaporphine over its S enantiomer vs. [^{3}H]APO binding.[14,34,35] A contamination of (S)-NPA by less than 1% (R)-NPA might be detectable in binding assays involving affinities in the nonomolar range. This possiblity was finally ruled out by our CD spectroscopic studies on the two enantiomers (Figure 1).

Additional support for the preferential effects of (R)-NPA on a marker of DA receptors was found in experiments with two preparations of CNS tissues containing DA-sensitive adenylate cyclase systems: homogenates of rat corpus striatum and intact pieces of the retina of a teleost fish (Table III and Figure 1). In both systems, the EC_{50} value for (R)-NPA (concentration producing half the maximum response) was approximately 1 μM, making it 100–1000 times more potent than (S)-NPA at stimulating adenylate cyclase activity.

The behavioral effects of NPA isomers were also consistent with the conclusion that the (R)-NPA is the preferred enantiomeric configuration to stimulate forebrain DA receptors in the rat, as is also proposed for APO.[13,19,35] Thus, we found that low doses of (R)-NPA induced strong stereotyped behavioral responses, while (S)-NPA was virtually incapable of inducing such presumed dopaminergic actions at doses up to 10 mg/kg sc (Table I). Moreover, the R isomer stimulated general motor activity (mainly a reflection of stereotyped behaviors). In contrast to (R)-NPA, high doses of the S enantiomer reduced general motor activity and induced moderate catalepsy

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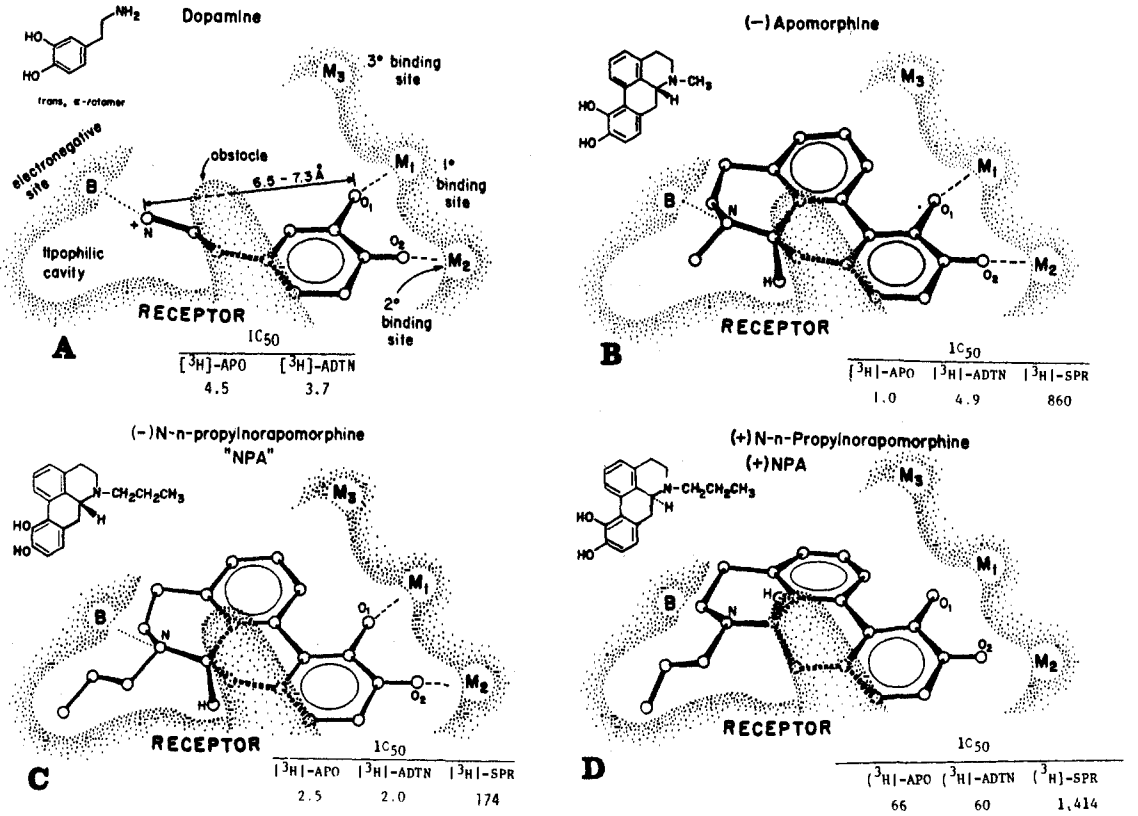

Figure 3. Interaction of dopamine (A), (*R*)-APO (B), (*R*)-NPA (C), and (*S*)-NPA (D) at the dopamine receptor. The salient features of this receptor model have been described previously.[17,35] The model accounts for the receptor-binding characteristics of aporphines and related rigid analogues of dopamine. The features include M_1 and M_2 putative binding sites on the receptor surface complementary to OH binding sites on the agonist molecule; an M_3 binding site that represents an interaction by OH substituents in position 2 of the A ring that *diminishes* affinity; and an electronegative site B complementary to a nitrogen atom on the agonist molecule. The optimal distance of the OH binding site complementary to M_1 is 6.5–7.3 Å from the nitrogen atom (trans α-rotameric conformation of dopamine). Substituents on the nitrogen should be accommodated by the lipophilic cavity on the receptor surface. A steric hindrance factor accounts for receptor boundaries (O-methylation reduces the potency of aporphine binding; 9,10-disubstitution on the aporphine ring prevents binding). Hydrogen atoms shown only at carbon 6a (chiral center) of aporphines accounts for enantioselectivity. Obstruction or obstacle on the receptor precludes the appropriate interaction in the 6a*S* configuraiton (Figure 3D). IC_{50} (nM) values were determined for calf caudate nuclei as for Table III or as discussed elsewhere.[25-27]

(Table I). These latter actions suggest that (*S*)-NPA may not only be inactive as a DA agonist but might also exert modest antagonistic effects on DA systems in the CNS. However, this latter suggestion was not borne out by direct competition between even high doses of (*S*)-NPA (5 mg/kg, sc) vs. the stereotyped gnawing responses to 3.0 mg/kg of (*R*)-NPA or (*R*)-APO, the approximate ED_{90} for stereotypy for each in the rat (see Table II). This inactivity on direct competition is, for uncertain reasons, apparently not in accord with observations reported by Riffee et al.,[13] which indicated an antagonistic behavioral interaction of (*S*)-APO with its dopaminergic *R* enantiomer in the mouse.

Our observation that the *S* isomer of NPA had a slightly higher affinity for COMT, while it was no more easily methylated than (*R*)-NPA at saturating concentrations of substrate, indicates that COMT is little influenced by stereochemical differences at the 6a chiral carbon atom of NPA. This position corresponds to the α position of the ethylamine side chain of catecholamines, such as DA. While there are some indications that COMT activity is altered by α or β substitution of catecholamines,[36-38] isomeric effects of such substitutions have generally been small or negligible.[37,38] Our observations with NPA appear to be the first demonstration of stereoselectivity, albeit small, by COMT for a catechol aporphine. Possibly, the apparent lack of strong central pharmacological activity of (*S*)-NPA, coupled with its somewhat greater affinity for COMT, can be exploited in the development of agents, such as COMT inhibitors or other potentiators of L-Dopa, much as has been done with polar derivatives of Dopa or other catechols that act as selective peripheral inhibitors of COMT or Dopa decarboxylase.[38,39]

An additional implication of the present results is that the isomeric difference between (*R*)- and (*S*)-NPA, with respect to DA receptors in the various tests used, adds to the impression that the 6a(*R*) configuration of D-ring hydroxylated aporphines is required for optimal attachment at CNS DA-receptor sites. A DA "receptor" model that we have advanced[17,34,35] can only be accommodated by the *R* configuraiton of NPA or APO (Figure 3B,C).

The structural requirements of such a schematic receptor (Figure 3) have emerged as a result of our evaluation of a wide variety of aporphine congeners and structurally related dopamine agonists. Putative sites of interaction with DA agonists are indicated in Figure 3A–C in which

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(40) The configuraiton of the chiral center of (−)-5,6-ADTN is designated as *S* even though this compound has the same projection of the hydrogen atom relative to the catechol ring, as in (*R*)-APO and (*R*)-NPA. Similarly, (+)-6,7-ADTN is designated as *R* and has the opposite projection of the hydrogen atom relative to the catechol ring as in (*R*)-APO and (*R*)-NPA.[16]

complementary binding sites coincide with the receptor surface, indicated as M_1, M_2, M_3, and B. Nitrogen atoms are shown as the free amines, though it is more likely that they are all protonated at physiological pH and interact with an electronegative site B on the receptor surface. An apparently critical feature in this[17,34,35] and other[19] DA agonist binding site models is the distance of 6.5–7.3 Å between the nitrogen atom and the *m*-hydroxy moiety of DA (Figure 3A) or the corresponding 11-OH in D-ring hydroxylated aporphines, the 5-OH of (*S*)-(−)-2-amino-5,6-dihydroxy-1,2,3,4-tetrahydronaphthalene[40] [(−)-5,6-ADTN], and the 6-OH of (*R*)-(+)-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene[40] [(+)-6,7-ADTN] indicated by M_1 in the receptor model in Figure 3. In order to account for the inactivity of (±)-9,10-dihydroxyaporphine (isoapomorphine) it has been suggested by McDermed and Freeman[16] and others[18] that the A ring of isoapomorphine sterically hinders attachment to the receptor principally at the M_1 and M_2 putative binding sites. However, in the case of 6,7-ADTN the more active *R*-(+) isomer can fit on the receptor without interference of the extra phenyl ring present in aporphines. Comparison of the *S* isomer of isoapomorphine in our receptor model with (+)-6,7-ADTN precludes an appropriate fit due to the interference of the receptor boundary. The observations[15,34,35] that O-methylation at the 10 or 11 position of aporphines considerably reduces the potency of binding brought about by the bulk of additional methyl group(s), thus precluding an appropriate fit on the receptor, further supports the current hypothesis for the structural requirements of this receptor model.

It should also be noted that in the *R* series of active aporphines, substitution of a 2-OH, as in 2,10,11-trihydroxyaporphines, led to *diminished* potency of binding. Such substituents on the A ring of aporphines may interfere by interacting at this region (M_3) of the receptor surface.[14,35] The electronic and steric effects of the substituents at this position remain to be clarified and are the focus of our current studies. A second and equally important region of steric interference, particularly relevant for the 10,11-dihydroxyaporphines, is the region shown in Figure 3 as an "obstacle". This region of steric interference would thus account for the stereoselectivity of the dopaminergic aporphines and the aminotetralins. The enantioselectivity of (*R*)-NPA suggests a specific effect resulting from the interaction of such molecules with complementary binding sites of a receptor surface.

Experimental Section

General Methods. All melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. Thin-layer chromatography (TLC) used recoated silica gel 13179, polyethyleneterphthalate sheets (Eastman Kodak, Rochester, NY). Ultraviolet spectra were recorded in acetonitrile on a Perkin-Elmer Lamda 5 spectrophotometer. Circular dichroism spectra were recorded in acetonitrile on a JASCO Model 500C CD spectropolarimeter. Optical rotations were obtained on a Perkin-Elmer polarimeter (Model 241).

Resolution of 10,11-Dimethoxy-*N*-*n*-propylnoraporphine [(*R*)- and (*S*)-3]. To a solution of 6.0 g (19 mmol) of (±)-10,11-dimethoxy-*N*-*n*-propylnoraporphine [(*RS*)-3][22] dissolved in 35 mL of EtOAc was added a solution of 7.0 g (190 mmol) of (+)-dibenzoyl-D-tartaric acid [Sigma Chemical Co.; $[\alpha]^{22}_D$ +124° (*c* 1.2, EtOH)] dissolved in 35 mL EtOAc containing 2 mL of 2-propanol. The mixture was heated to reflux for 10 min and then filtered and cooled. After the mixture was left standing at 0 °C for 3 days, 3 g of gray crystals was collected. This (+)-dibenzoyl-D-tartrate salt was crystallized three times from a mixture of 30 mL of EtOAc, 15 mL of 2-propanol, and 15 mL of EtOH: mp 154–155 °C; yield 0.5 g. Further recrystallizations did not change the specific rotation: $[\alpha]^{22}_{578}$ +115.0° (*c* 0.2, MeOH).

The crystalline salt was dissolved in aqueous saturated Na_2CO_3 solution, and the mixture was extracted with $CHCl_3$. After drying (Na_2SO_4), the extract was converted into the hydrochloride salt to give 336 mg of an off-white powder of (*S*)-3·HCl: mp 110–115 °C; $[\alpha]^{22}_D$ +89.6° (*c* 0.18, MeOH).

The combined mother liquors containing the (*R*)-(−)-3 salt were treated with aqueous saturated Na_2CO_3 solution to liberate the free base, extracted with EtOAc, dried, and evaporated to dryness. The remaining 5.5 g of oil was dissolved in 35 mL of EtOAc and treated with 6.6 g (18 mmol) of (−)-dibenzoyl-L-tartaric acid [Sigma Chemical Co.; $[\alpha]^{22}_D$ −121.6° (*c* 1.5, EtOH)] and treated as described above to give 1.0 g of the (*R*)-3 salt, mp 154–155 °C; $[\alpha]^{22}_{578}$ −114.64° (*c* 0.25, MeOH). The crystalline salt was dissolved in aqueous saturated Na_2CO_3 solution, and the mixture was extracted with $CHCl_3$. After drying (Na_2SO_4), the extract was converted to the hydrochloride salt to give 0.43 g of (*R*)-(−)-3·HCl: mp 112–116 °C; $[\alpha]^{22}_D$ −91.7° (*c* 0.22, MeOH).

(*RS*)-*N*-*n*-Propylnorapomorphine hydriodide was prepared via a multistep synthesis by a method previously described.[22]

(*S*)-*N*-*n*-Propylnorapomorphine Hydrochloride [(*S*)-2·HCl]. To 0.3 g (0.97 mmol) of (*S*)-3·HCl dissolved in 10 mL of glacial AcOH was added 10 mL of HBr (48%, w/v). The reaction mixture was heated under an atmosphere of N_2 at reflux in an oil bath for 4 h and then allowed to cool under nitrogen. Monitoring of the mixture by TLC indicated that complete ether cleavage had occurred. To the cooled (ice bath) reaction mixture was added 100 mg of $NaHSO_3$. The pH of the solution was adjusted to 8 with aqueous Na_2CO_3 solution, and the liberated free base was extracted from $CHCl_3$ and converted to the hydrochloride salt: yield 235 mg (76%) of (*S*)-(+)-2·HCl; mp 178–180 °C; $[\alpha]^{22}_D$ +62.05° (*c* 0.166, MeOH); UV λ_{max} 274 nm with a broad shoulder at 312 nm. (*R*)-(−)-2·HCl, obtained by the rearrangement of 1, gave $[\alpha]^{22}_D$ −63.21° (*c* 0.166, MeOH).

Racemization of (*R*)-10,11-Dimethoxy-*N*-*n*-propylnoraporphine. (*R*)-3 (3.5 g, 11 μmol) in 160 mL of CH_3CN was allowed to reflux with 3.2 g of 10% Pd on charcoal under an atmosphere of nitrogen. After 12 h, TLC indicated complete conversion to the dehydroaporphine (4). The catalyst was filtered off, and the filtrate was evaporated to give a green oil; this was dissolved in 420 mL of absolute EtOH, and 3.5 g (55 mmol) $NaBH_3CN$ was added; EtOH/HCl was added until the pH was 5.0. The pH was maintained over 24 h by several additions of the EtOH/HCl. After evaporation of the reaction mixture, the pH was adjusted to 8 with saturated aqueous Na_2CO_3 solution, and the free base was extracted from $CHCl_3$ as 2.8 g of a yellow oil: $[\alpha]^{22}_D$ 0° (*c* 0.24, MeOH). The oil was converted to [(±)-3·HCl], mp 235–236 °C dec. The demethylation of (±)-3·HCl with HBr was carried out as described above and gave (±)-2·HCl, mp 258–259 °C dec.

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Registry No. 1, 41590-60-7; (±)-2, 57559-68-9; (±)-2·HCl, 41649-44-9; (*R*)-(−)-2, 18426-20-5; (*R*)-(−)-2·HCl, 20382-71-2; (*S*)-(+)-2, 79703-31-4; (*S*)-(+)-2·HCl, 84454-80-8; (±)-3, 84454-81-9; (±)-3·HCl, 50370-80-4; (*R*)-(−)-3, 84579-75-9; (*R*)-(−)-3·HCl, 84579-76-0; (*R*)-(−)-3 (+)-dibenzoyl-D-tartrate, 84579-77-1; (*R*)-(−)-3 (−)-dibenzoyl-L-tartrate, 84579-78-2; (*S*)-(+)-3·HCl, 84579-79-3; (*S*)-(+)-3 (+)-dibenzoyl-D-tartrate, 84579-81-7; 4, 84454-82-0.